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Lipid Exchange Rates of Conventional and Polymer Stabilized Liposomes

Awad Ahmed, Nicole Heldt, Gregory Slack, and Yuzhuo Li
Department of Chemistry and Center for Advanced Materials Processing,
Clarkson University, Potsdam, New York, 13699-5810, USA

ABSTRACT

Polymer-stabilized liposome systems consisting of polyethylene glycol bound lipids (PEG-lipids) and conventional (nonpolymer stabilized) liposomes were compared in terms of their inter-membrane lipid migration rates. In order to monitor the exchange of lipids between the membranes, 1-hexadecanoyl-2-(1-pyrenedecanoyl)-sn-glycero-3-phosphocholine (PY-PC), a phospholipid with pyrene attached to the hydrophobic tail, was used to label the liposome. Labeled and unlabeled liposome systems were mixed and fluorescence spectroscopy was used to examine the lipid transfer. More specifically, the relative employed to deduce the exchange kinetics. After labeled and unlabeled liposome systems were mixed, the E/M ratio for PY-PC in a polymer stabilized liposome system decreased by 66% over a period of 80 minutes, while the E/M for PY-PC in a conventional liposome system decreased 70% in less than 2 minutes. This suggests that the exchange rate for lipids in polymer stabilized liposome systems is much slower than that of conventional liposome systems. In addition, the exchange rates for both conventional and polymer stabilized liposome systems are accelerated at an elevated temperature.

INTRODUCTION

Liposome is a very important supermolecular structure because of its similarity to biological membranes and its therapeutic value as delivery agent for enzymes, drugs, genetic manipulation, and diagnostic imaging applications [1]. Recently, liposomes with polyethylene glycol chains attached to the polar head group (polymer stabilized or Stealth liposomes) have been implemented in pharmaceutical applications due to their increased lifetime in the blood stream [1,2].

In the field of liposome studies, fluorescence has emerged as a useful analytical technique, as it provides a large amount of diversified information concerning the biophysics of lipid vesicles and biomembranes [3]. Artificial and biological membranes can be considered as two-dimensional fluids, characterized by a high lateral mobility of lipid components. Almgran was the first to use a fluorescence stopped-flow technique to investigate the migration of pyrene between unilamellar vesicles [4]. The results indicated that the migration of pyrene form one vesicle to another occurs mainly via a desolubilizations-diffusion-resolubilization mechanism [4]. Doody et al. also provided evidence that the transfer of a fluorescent fatty acid probe is through the aqueous phase [5]. Sengupa et al. measured pyrenyl-decanoic acid transfer rates between DPPC vesicles using a stop-flow technique and found that multilamellar vesicles had much slower exchange rates than that of unilamellar vesicles. In addition, charging the surface of the vesicles also reduces the exchange rate [6]. Wolkowicz and collaborators found that an increase in the vesicle size reduces the transfer rate of a probe. Furthermore, natural membranes, such as cardiac and mitochondrial membranes, exhibit a marked decrease in transfer rates [7]. In a study by Ollmann et al., the lateral lipid diffusion rate in a phosphatidylcholine membrane was determined by using pyrene-labeled gangliosides as probes [8]. It was found that the lateral diffusion rate is signigficantly higher than the inter-membrane transfer rate.

In addition to conventional liposome systems, inter-membrane lipid transfer between polymer-stabilized liposomes has also been studied. In one investigation, the transfer of PEG-lipids was monitored using biotinylated (acceptor) liposomes [9]. When PEG-liposomes were mixed with biotinylated liposomes, the inhibition of the biotinylated liposomes to bind to streptavidin was used to measure the transfer of the PEG-lipids [10]. It was found that transfer of PEG-lipids is dependent upon temperature and acyl chain length of the lipid anchor. Lipid transfer is decreased when the acyl chain length is increased and increased with increases in temperature [9].

A systematic study and comparison between the properties of conventional and polymer-stabilized liposomes are fundamentally important to the application of vesicles in drug delivery. The information is critical for a structural design that balances the need for polymer stabilization or "stealth effect" and the specificity possessed by the original membrane surface. It is desirable to develop a liposome system that has an adequate stealth effect without compromising the surface activity that is critical in targeting other membranes for drug delivery. The use of polymer stabilized liposomes as ligand targeting agents is still somewhat limited because the protective polymer border prevents most molecules from being attached [10]. The focus of this paper is the investigation of lipid exchange rate difference between conventional and polymer stabilized liposome systems.. The lipid transfer rates were monitored using a fluorescent labeled phospholipid, 1-hexadecanoyl-2- [1-pyrenedecanoyl)-sn-glycero-3-phosphocholine (PY-PC). The temperature effect on lipid exchange rates was also investigated

EXPERIMENTAL DETAILS

Materials

The lipids used were 1,2-dipalmitoyl-sn-glycero-3-phosphate monosodium (DPPA) (Avanti Polar Lipids, Inc. Alabaster, AL), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) (Lipoid GmbH, Ludwigshafen, Germany), 1,2-Dipalmioyl-sn-glycero-3-phosphoethanolamine-N- [methoxypolyethyleneglycol 5000] (DPPE-MPEG 5000) (Shearwater Polymers, Inc Huntsville, AL), 1,2-dipalmioyl-sn-glycero-3-phosphoethanolamine (DPPE) (Sygena, Inc. Cambridge, MA), and the pyrene labeled phospholipid, 1-hexadecanoyl-2- (1-pyrenedecanoyl)-sn-glycero-3-phosphocholine (PY-PC) (Molecular Probes, Inc. Eugene, OR). Extrusion was accomplished by the Mini-Extruder® (Avanti Polar Lipids, Inc. Alabaster, AL).

Liposome Preparation

Polymer stabilized liposome systems were prepared by adding a 9:1:1 molar blend of DPPC, DPPA, and DPPE MPEG-5000 to polyethylene glycol and bath sonicating at 69°C until clear. A mixture of 1 part glycerol to 8 parts 0.9% aqueous NaCl was then added to the sonicated blend. The conventional liposomes were prepared in the same manner as the polymer stabilized liposome systems except that the DPPE-MPEG 5000 was replaced by DPPE. In addition, the conventional liposome samples were extruded through 0.1 polycarbonate filters. Pyrene-labeled liposome systems of polymer stabilized and conventional liposomes were prepared by adding 1 mg of PY-PC (dissolved in 1ml ethanol) to 75 mg of dry lipid blends.

Fluorescence Emission Spectra

All samples were purged with and sealed under nitrogen gas to prevent oxygen quenching. All monomer and excimer intensity measurements were performed on a Perkin Elmer LS 50 spectrophotometer. The excitation wavelength was set at 340nm (2.5nm slit width). Monomer and excimer emission peaks were observed at 395nm and 476nm, respectively. Mixtures of 1:1 pyrene-labeled and unlabeled liposome systems for both polymer stabilized and conventional liposomes were examined at various temperatures (12°C, 24°C, 37°C, and 50°C). PY-PC transfer between liposome membranes was determined by the decrease in E/M after labeled and unlabeled liposome systems were mixed.

Particle Size

Particle size and size distribution were measured using a dynamic light scattering (DLS) method, which was performed on a Brookhaven Instruments System, equipped with a water-cooled argon-ion laser light source (Model 85, Lexel Laser). The device was operated at the wavelength of 514.5nm and a 90 degree of scattering angle.

RESULTS AND DISCUSSION

Conventional and polymer stabilized liposome systems were prepared and characterized as described in the experimental section. Dynamic Light Scattering (DLS) measurements show that conventional and polymer stabilized liposome systems have average particle sizes ranging from 100 to 200nm. Although both systems give a similar particle size range, it is understood that the PEG chains attached to the lipid bilayer of polymer-stabilized liposomes contribute to a significant share of the particle size.

Conventional and polymer stabilized liposome systems containing a fluorescence probe were prepared according to the procedures described in the experimental section. Pyrene labeled 1-hexadecanoyl-2-(1-pyrenedecanoyl)-sn-glycero-3-phosphocholine (PY-PC) has the pyrene molecule attached to the fatty acid tail end, which is located in the lipid bilayer region. Fluorescence emission spectra were obtained for both systems as well. The emission at 395 nm is assigned to one of the monomer peaks (M) and 476 nm to the excimer peak (E). Peak ratios E/M were then calculated and used to study the migration of pyrene-labeled lipids from one liposome (labeled) to another (unlabeled) [11]. There are two possible modes for the migration of the probe from vesicle to vesicle. PY-PC may either migrate via the aqueous phase, or move from one vesicle to another during vesicle collision [3,9].

Excimers may form upon collision between an excited pyrene derivative and a ground state pyrene derivative [12]. The E/M ratio is thus proportional to the collision frequency of pyrene derivatives, which in turn depends on the local concentration and lateral diffusion rate of pyrene derivatives [11]. A greater E/M ratio indicates a greater local concentration of PY-PC. L'Hereux and Fragata studied the localization of pyrene and pyrenyl-hexadecanic acid in PC small unilamellar vesicles [13]. It was found that below 4 molar percent in probe molecules the excimer formation is essentially diffusion controlled, while above 4% excimers are obtained in aggregated form. In our study, the molar percent of the fluorescent probe is significantly lower than 4%. The excimer formation is, therefore, likely to be a diffusion controlled process. Interestingly, the conventional liposomes yielded E/M values nearly two times greater than

polymer stabilized liposome systems. (Figures 1, 2 and Table 1) The larger E/M value for conventional liposome systems is a strong indication that the lateral diffusion rate is significantly higher than that in a polymer stabilized system.

The effect of temperature on excimer formation in both conventional and polymer-stabilized liposomes was also examined. As seen in Figure 1 and Table 1, the higher the temperature smaller the initial excimer peak intensity. As temperature increases, the mobility of the lipid molecules increases and the excimer lifetime decreases. Conventional liposome systems appeared to be more sensitive to temperature changes than polymer stabilized liposome systems, as indicated by a larger drop in excimer emission from 24 to 50°C (Table 1). This is again consistent with the notion that the PEG chains reduce the mobility of the lipids in the polymer stabilized liposome bilayers [14]. In addition, PEG produces a shielding effect that not only prevents molecules from binding the lipid surface, but also restricts the molecules within the lipid bilayer from leaving.

Roseman and Thompson used a pyrene labeled phosphatidylcholine to study the kinetics of spontaneous phospholipid transfer between unilamellar (conventional) liposomes [15]. It was found that there is a significant rate decrease for phospholipid transfer when the flip-flop rate exceeds the lipid migration at certain temperature. It was also concluded that pyrene-PC transfer takes place through the aqueous medium the than collision of membranes, and is independent of acceptor vesicle concentration [15].

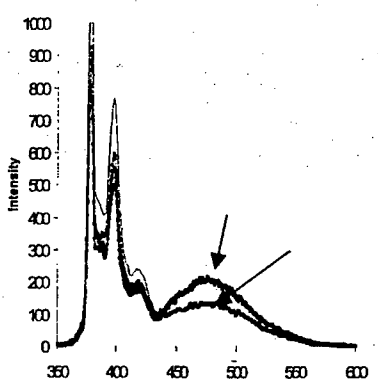

Figure 1. Effect of temperature on excimer formation in polymer stabilized liposome systems.

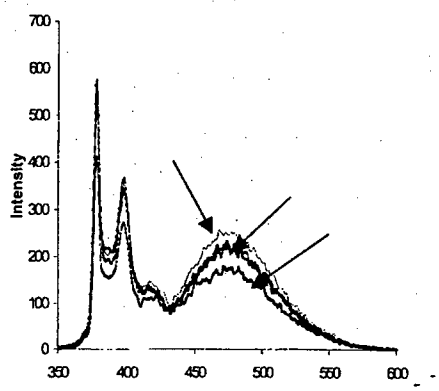

Figure 2. Effect of temperature on excimer peak formation in conventional liposome systems.

Table 1: Effect of temperature on excimer formation in conventional and polymer-stabilized liposomes (monomer peaks measured at 395 nm and excimer peaks measured at 476 nm)

Temperature °C	Conventional Liposomes			Polymer Stabilized Liposomes		
	Monomer (M)	Excimer (E)	E/M	Monomer (M)	Excimer (E)	E/M
12	358.8	251.6	0.70	764.4	210.0	0.27
24	302.8	221.5	0.73	580.2	210.0	0.36
37	266.9	224.4	0.84	495.1	210.0	0.42
50	360.2	177.0	0.49	563.2	131.7	0.23

In our study, as shown in Figures 1 and 2, there is a gradual change in initial E/M ratios for conventional liposome systems and a sharp change above 37 °C for polymer stabilized liposome systems. It is possible that the temperature at which the flip-flop rate is greater than inter-membrane transfer for polymer stabilized liposome systems is somewhat greater than that for a conventional liposome. This is a direct result of the rigidity introduced by the PEG chains.

The impact of PEG chains on inter-membrane lipid transfer was further examined and illustrated in Figure 3. As an inter-membrane lipid exchange should result in a decrease in E/M ratios, a plot of E/M vs time represents the exchange kinetics. When a labeled conventional liposome sample was mixed with an unlabeled conventional liposome sample, the E/M ratio decreased ca. 70% in less than two minutes (not shown), followed by a very slow exchange process in agreement with Galla et al [16]. When a labeled polymer stabilized system was mixed with another polymer stabilized system, the E/M ratio decreased ca. 60% in 80 minutes. When a labeled conventional liposome sample was mixed with an unlabeled polymer stabilized liposome sample, the initial exchange behavior is similar to the conventional-conventional case, followed by an E/M decrease that is parallel to the polymer-polymer case.

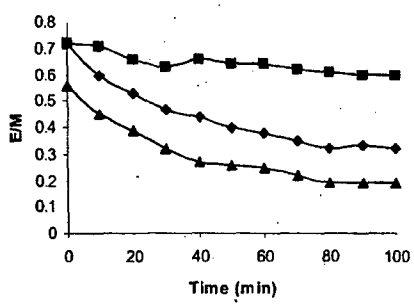

Figure 3. Lipid exchange (E/M) for mixtures of labeled conventional and unlabeled polymer stabilized liposomes (-◆-) labeled and unlabeled polymer stabilized liposomes (-▲-) and labeled and unlabeled conventional liposomes (-■-).

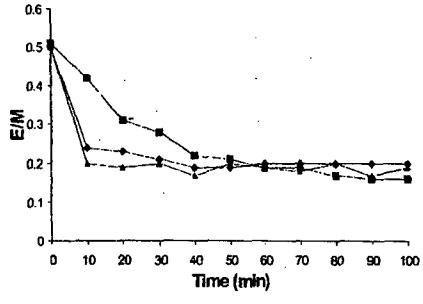

Figure 4. Lipid exchange (E/M) for mixtures of PY-PC labeled and unlabeled polymer stabilized at different temperatures (24°C -■-, 37°C -◆-, and 50°C -▲-).

The remarkable difference between polymer stabilized and conventional liposome systems in lipid exchange rate can be traced to the shielding effect of the PEG border and the rigidity of the lipid bilayer due to the presence of PEG-lipids. The temperature dependence of the exchange rate in polymer-stabilized liposome is shown in Figure 4. As expected, an increase in temperature directly corresponded to an increase in lipid exchange rate. Temperature effect found in this study for polymer stabilized liposome system is consistent with findings reported for conventional liposome systems [17].

CONCLUSIONS

Changes in E/M values can be used to monitor the exchange rate of lipids in conventional liposome systems and polymer stabilized liposome systems. Polymer stabilized liposome systems were observed to have slower lipid exchange rates than conventional liposome systems. In addition, polymer stabilized liposome systems had a lower sensitivity to temperature changes. It is believed that the PEG chains that form the outer portion of the lipid bilayer for these polymer-stabilized liposomes provides restricted mobility of the lipids within the lipid bilayer and the interliposomal phospholipid transfer.

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